AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Development and Validation of UV Spectrophotometric Method for Simultaneous Estimation of Lamivudine, Didanosine and Efavirenz in the Pharmaceutical Dosage Form

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ABSTRACT

A rapid, simple, sensitive, accurate, and precise UV spectrophotometric method has been developed for the simultaneous estimation of anti-retroviral agents lamivudine, didanosine and efavirenz in pharmaceutical dosage form. The absorption maxima of the drugs were found to be 271, 250 and 247 nm for lamivudine, didanosine and efavirenz respectively. Lamivudine, didanosine and efavirenz obeyed Beer's law in the concentration range of 10-100 µg/ml, 10-100 µg/ml and 10-70 µg/ml respectively. The percentage recovery was within the range of 98% - 101%, indicating that insignificant interference from the other ingredients in the formulation. The above method was validated in terms of linearity, accuracy, precision, Limit of Detection (LOD), Limit of Quantification (LOQ) etc. in accordance with ICH guide lines. The developed method was free from interferences due to excipients present in tablets. The method was rapid, simple and suitable for routine quality control analysis.

Keywords: lamivudine; didanosine; efavirenz; UV spectrophotometric method; simultaneous equation method

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Received 15 October 2012, Accepted 23 October 2012

Please cite this article in press as Sandhya BN *et al.*, Development and Validation of UV Spectrophotometric Method for Simultaneous Estimation of Lamivudine, Didanosine and Efavirenz in the Pharmaceutical Dosage Form. American Journal of PharmTech Research 2012.

INTRODUCTION

Nucleoside reverse transcriptase inhibitors (NRTIs) were the first class of drugs, which were introduced as antiretroviral agents for the treatment of infection with human immune deficiency virus (HIV). Additional drug classes were developed. They are protease inhibitors (PIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs) and fusion inhibitors.

Lamivudine is nucleoside reverse transcriptase inhibitors with activity against human immune deficiency virus (HIV) and hepatitis B virus Figure. 1(A). Nucleoside reverse transcriptase inhibitors (NRTIs) are the prodrugs that require intracellular phosphorylation to their corresponding triphosphate derivatives, which are the active inhibitors of HIV reverse transcriptase [1, 2]

Didanosine is nucleoside reverse transcriptase inhibitor with activity against human immune deficiency virus (HIV) and hepatitis B virus Figure. 1(B). It acts as a chain terminator of viral DNA [3, 4.]

Efavirenz is non-nucleoside reverse transcriptase inhibitors. It is used in the treatment of HIV infection Figure. 1(C). It binds directly and reversibly to the catalytic site of the reverse transcriptase enzyme [5, 6]

According to the literature survey it was found that few analytical methods such as UV [(7-9)] and HPLC methods [(10-14)] were reported for lamivudine, didanosine and efavirenz. To our knowledge, no study related to the UV spectrophotometric method for the simultaneous estimation of lamivudine, didanosine and efavirenz have been reported in literature. Therefore, there is a challenge to develop UV Spectrophotometric method for the simultaneous estimation of lamivudine, didanosine and efavirenz. The present study was involved in a research effort aimed at developing and validating a simple, specific, accurate, economical and precise UV spectrophotometric method for the simultaneous estimation of three drugs in pharmaceutical dosage form.

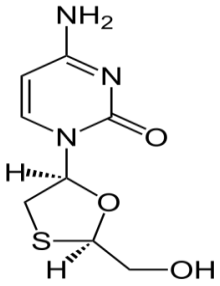

Figure. 1a. Structure of Lamivudine

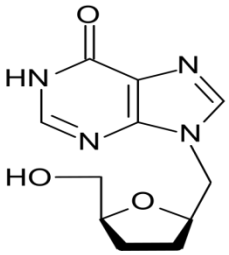

Figure. 1b. Structure of Didanosine

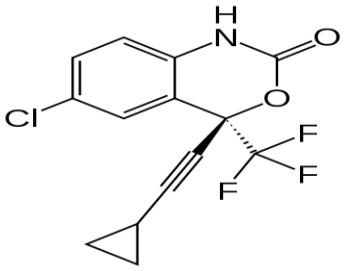

Figure. 1c. Structure of Efavirenz

MATERIALS AND METHOD

Materials:

Hydrochloric acid, Sodium hydroxide, water, acetonitrile, methanol, lamivudine, didanosine and efavirenz.

Instrument:

UV double beam spectrophotometer (Shimadzu model 1800) was employed with a spectral band width of 1nm and a wavelength accuracy of 0.3 nm (with automatic wavelength correction with a pair of 1 cm matched quartz cells**).**

Method:

The UV spectra's of lamivudine, didanosine and efavirenz in different solvents like water, acetonitrile, methanol, sodium hydroxide and hydrochloric acid were recorded. These three drugs showed good absorbances when dissolved in acetonitrile. Hence acetonitrile was selected as the solvent for the method. Lamivudine, didanosine and efavirenz (10 mg each) were separately weighed and transferred to a 100 ml volumetric flask and all the three drugs were dissolved in acetonitrile to get a solution of 100 µg/ml. Working standard solutions of 50 µg/ml of each of the drugs were prepared and scanned in the range 400-200 nm to obtain the absorbance spectra and overlain spectra (Fig.2). Three wavelengths 271, 250 and 247 nm were selected which are the λmax of three drugs lamivudine, didanosine and efavirenz respectively. The absorbance of lamivudine, didanosine and efavirenz was measured and the absorptivity values E (1%, 1cm) were determined at all the three selected wavelengths. The concentrations of three drugs in mixture can be calculated using the following equations,

$C_{lamivudine}$ = (A1(ay2az3-az2ay3)-ay1(A2az3-az2A3)+az1(A2ay3-ay2A3)/ax1(ay2az3-az2ay3)-ay1(ax2az3-az2ax3)+az1(ax2ay3-ay2ax3) (1)

$C_{didanosine}$ = (ax1(A2az3-az2A3)-A1(ax2az3-az2ax3)+az1(ax2A3-A2ax3)/ax1(ay2az3-az2ay3)-ay1(ax2az3-az2ax3)+az1(ax2ay3-ay2ax3) (2)

$C_{efavirenz}$ = (ax1(ay2A3-A2ay3)-ay1(ax2A3-A2ax3)+A1(ax2ay3-ay2ax3)/ax1(ay2az3-az2ay3)-ay1(ax2az3-az2ax3)+az1(ax2ay3-ay2ax3) (3)

Where, $C_{lamivudine}$, $C_{didanosine}$ and $C_{efavirenz}$ are the concentrations of lamivudine, didanosine and efavirenz respectively in mixture and in sample solutions. A_1, A_2 and A_3 are the absorbances of sample at 271, 250 and 247 nm, respectively, ax_1, $ax_2$and ax_3 are the absorptivity of lamivudine at 271, 250 and 247 nm respectively, ay_1, ay_2 and ay_3 are the absorptivity of didanosine at 271, 250 and 247 nm, respectively, az_1, az_2 and az_3 are the absorptivity of efavirenz at 271, 250 and 247 nm, respectively.

RESULTS AND DISCUSSION:

The analytical method was validated with respect to parameters such as linearity, precision, accuracy, limit of detection (LOD), limit of quantitation (LOQ) and ruggedness.

Linearity:

Linearity was established by least squares linear regression analysis of the calibration curve. The calibration curves were linear over the concentration range of 10-100 µg/ml for lamivudine, 10-100 µg/ml for didanosine and 10-70 µg/ml for efavirenz. Absorbances were plotted versus respective concentrations and linear regression analysis was performed on the resultant curves. Correlation coefficients were found to be 0.996, 0.997 and 0.999 for lamivudine, didanosine and efavirenz respectively (Figure. 3-5). The results are given in Table 1.

Table 1: Linearity and correlation coefficient

Parameters	Lamivudine	Didanosine	Efavirenz
Regression equation	y = 0.020x + 0.030	y = 0.044x + 0.063	y = 0.057x - 0.004
Linearity µg/ml	10 – 100	10 – 100	10 - 70
Correlation coefficient	0.996	0.997	0.999

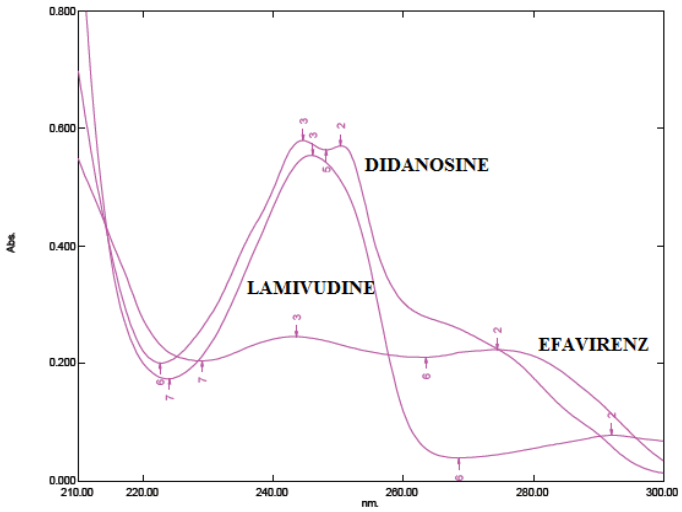

Figure. 2 Overlain spectra of lamivudine, didanosine and efavirenz

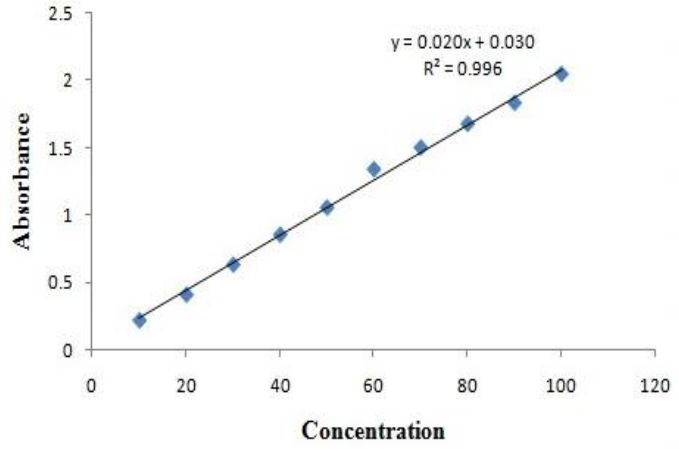

Figure. 3: Calibration Curve of Lamivudine at 271 nm

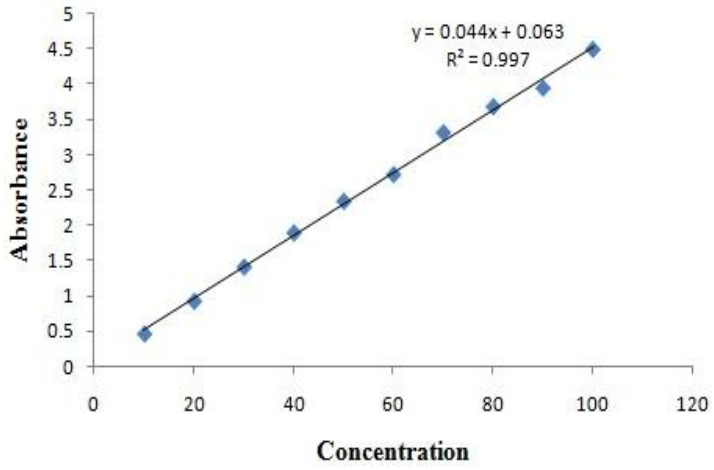

Figure. 4: Calibration Curve of Didanosine at 250 nm

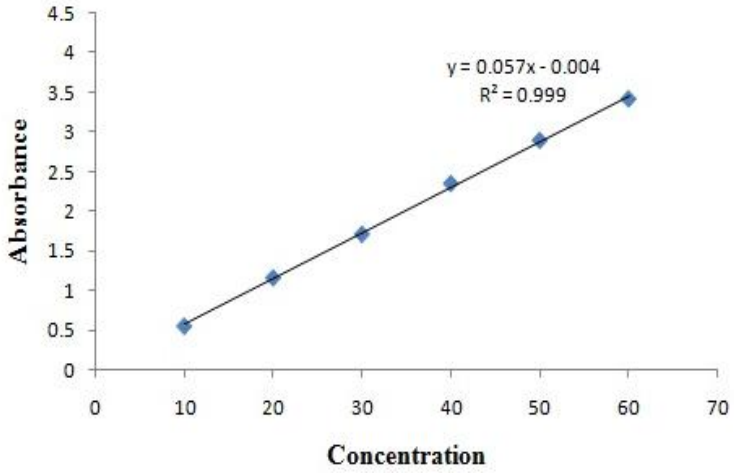

Figure. 5:Calibration Curve of Efavirenz at 247 nm

Precision:

To check the degree of repeatability of the method, suitable statistical evaluation was carried out. The concentrations of three drugs were measured three times on the same day at intervals of 1hr

and on three different days for intra and inter day study, respectively. The Standard Deviation (SD) and Relative Standard Deviation (RSD) were calculated. The results are given in Table 2.

Table 2: Precision studies

Drug	Concentration µg/ml	Intraday Precision (n=3)	Inter day Precision (n=3)
		% RSD	%RSD
Lamivudine	50	0.487	0.726
Didanosine	50	0.236	0.321
Efavirenz	50	0.172	0.356

Accuracy:

Recovery studies were carried out by applying the method to drug sample to which known amount of standard lamivudine, didanosine and efavirenz corresponding to 50, 100 and 150% of label claim had been added. At each level of the amount six determinations were performed. The results are given in Table 3.

Table 3: Accuracy

Drug	% Amount added	Amount taken(mg)	Amount recovered(mg)	% Recovery	% *RSD
Lamivudine	50	150	148	99.82	0.461
	100	300	298		
	150	450	450		
Didanosine	50	125	123	99.73	0.624
	100	250	248		
	150	375	372		
`Efavirenz	50	300	298	99.94	0.546
	100	600	597		
	150	900	898		

*mean of six observations

LOD and LOQ:

The LOD of lamivudine, didanosine and efavirenz was found to be 3.5 µg/ml, 3.5 µg/ml and 3.0 µg/ml respectively and the LOQ was found to be 10 µg/ml,10 µg/ml and 10 µg/ml respectively. The results are given in Table 4.

Table 4: LOD and LOQ studies

Validation parameters	Lamivudine	Didanosine	Efavirenz
(LOD) µg/ml	3.5	3.5	3.0
(LOQ) µg/ml	10	10	10

Analysis of marketed formulation:

Twenty Odivir-kits each containing 300 mg lamivudine, 250 mg didanosine, 600 mg efavirenz were weighed, average weight was calculated and powdered. A quantity equivalent to 300 mg of lamivudine, 250 mg of didanosine and 600 mg of efavirenz was weighed and transferred into 100

ml volumetric flask. It is extracted with acetonitrile. The volumetric flask was sonicated for 20 minutes to affect the complete dissolution of the drugs and the solution was made up to the volume with acetonitrile and filtered. Suitable aliquots of formulation were prepared and scanned to obtain concentration of the three drugs in the linearity range. The concentration of each analyte was determined using the simultaneous equation (Figure. 6) (Table 5).

Table 5: Analysis of Formulation

Drug	Labelled amount (mg/tablet)	Amount found (mg/tablet)	% Label claim	% *RSD
Lamivudine	300	290	99.67	0.095
Didanosine	250	248	99.2	0.122
Efavirenz	600	598	99.67	0.715

* mean of six observations

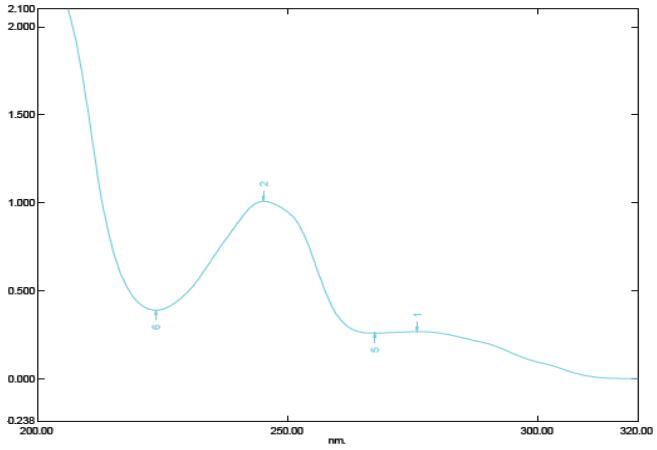

Figure. 6: Spectrum of Lamivudine, Didanosine and Efavirenz formulation

CONCLUSION:

This method is considered simple, reliable, selective providing satisfactory accuracy, precision with lower limits of detection and quantification more specific and sensitive. The good recoveries were obtained in all cases as well as the reliable agreement with the reported procedure proved that the proposed methods could be applied efficiently for determination of lamivudine, didanosine and efavirenz in oral dosage form with satisfactory precision. More over the shorter duration of analysis for lamivudine, didanosine and efavirenz makes the reported method suitable for routine analysis in pharmaceutical dosage forms.

ACKNOWLEDGEMENT

I am very much thankful to School of pharmacy, Anurag Group of Institutions, Hyderabad, for giving permission to carry out my research work.

REFERENCES

1. en.wikepedia.org/wiki/lamivudine accessed on 1-1-2012.
2. http://www.drugbank.ca/drugs/DB00709 accessed on 1-1-2012.
3. en.wikipedia.org/wiki/didanosine accessed on 1-1-2012.
4. www.drugbank.ca/drugs/DB00900 accessed on 1-1-2012.
5. en.wikipedia.org/wiki/efavirenz accessed on 1-1-2012.
6. www.drugbank.ca/drugs/DB00625 accessed on 1-1-2012.
7. Deepali G,Elvis M. UV spectrophotometric method for assay of the anti-retroviral agent lamivudine in active pharmaceutical ingredient and in its tablet formulation. Pharm analysis 2010; 2(4): 417-419.
8. Jaiprakash N, Sangshetti Parag A, Kulkarni and Devanand B. Shinde. Spectrophotometric determination of didanosine in bulk and tablet formulation. Trends in Applied Sciences Res 2007*;* 2(1): 71-75.
9. Anand Kumar Y, Rama Rao N. Development of rapid UV Spectrophotometric method for the estimation of efavirenz in formulations. E-J Chemistry 2010; 7(3): 856-860.
10. Bahrami G, Mirzaeei S, Kiani A, Mohammadi B. High-performance liquid chromatographic determination of lamivudine in human serum using liquid-liquid extraction application to pharmacokinetics. J Chromat. B 2005; 823: 213–217.
11. Kapoor N, Sateesh K, Ramesh P. Simultaneous determination of lamivudine and stavudine in antiretroviral fixed dose combinations by first derivative spectrophotometry and high performance liquid chromatography. J Pharma Biomed Ana 2006; 41*:* 761–765.
12. Mogatle S, Kanfer I. Rapid method for the quantitative determination of efavirenz in human plasma. J. Pharma Biomed Anal 2009; 49: 1308–1312.
13. Notari S, Mancone C, Alonzi M, Tripodi, Narciso P, Ascenzi. P. Determination of abacavir, amprenavir, didanosine, efavirenz, nevirapine, and stavudine concentration in human plasma by MALDI- TOF/TOF. J Chromat B 2008; 863: 249–257.
14. Notari S, Bocedi A, Ippolito G, Narciso P, Pucillo LP, Tossini G, Donnorso RP, Gasparrini F, Ascenzi P. Simultaneous determination of 16 anti-HIV drugs in human plasma by high-performance liquid chromatography. J Pharma Biomed. Anal 2006; 831: 258–266.